


# Plasmids

SK Seokhwi Kim

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 An abbreviated version of this protocol was published in Science Advances in Apr 2020

Dynamic Fas signaling network regulates neural stem cell proliferation and memory enhancement

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## Detailed protocol

We used pEGFP-N1 (<https://www.addgene.org/vector-database/2491/>) as a backbone vector. Lyn, cytoplasmic domain of FAS and PHR domain were sequentially inserted to the backbone vector by restriction enzyme cloning.

First, Lyn sequence was PCR-amplified from optoFGFR1 (<https://www.addgene.org/59776/>) by using the following primers and inserted to pEGFP-N1 with NheI/XhoI digestion to construct Lyn-EGFP: 5'-GTAGCTAGCCACC ATGGGATGTATAAAATCAAAGG-3' (forward) and 5'-GTACTCGAGCGCACTACCAGCACTACCAG-3' (reverse).

Then, cytoplasmic domain of FAS was PCR-amplified from HeLa cell cDNA by using following primers and inserted to Lyn-EGFP with XhoI/EcoRI digestion to construct Lyn-cyFAS-EGFP: 5'-GTACTCGAGAAGAGAAAGGAAGTACAGAAAACATGCAGA-3' (forward) and 5'-GTAGAATTCTGACCAAGCTTTGGATTTCATTT-3' (reverse).

Finally, PHR domain was PCR-amplified from optoFGFR1 (<https://www.addgene.org/59776/>) by using the following primers and inserted to Lyn-cyFAS-EGFP with BamHI/AgeI digestion to construct Lyn-cyFAS-PHR-EGFP (optoFAS): 5'-GTAGGATCCCATGAAGATGGACAAAAAGACCA-3' (forward) and 5'-GTAACCGGTGCGTACACGGCAGCACCGATC-3' (reverse).

**How to cite:** (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Kim, S. (2021). Plasmids. Bio-protocol Preprint. [bio-protocol.org/prep783](https://bio-protocol.org/prep783).
2. Kim, S., Kim, N., Lee, J., Kim, S., Hong, J., Son, S. and Heo, W. D.(2020). Dynamic Fas signaling network regulates neural stem cell proliferation and memory enhancement . Science Advances 6(17). DOI: [10.1126/sciadv.aaz9691](https://doi.org/10.1126/sciadv.aaz9691)

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